

A' com. (m) collecting the pellet from step (I) and lyophilizing the obtained pellet to store at 1

to 4° C for longer use.

Remarks

Claims 1-3, and 5-8 remain pending in the application. Claim 2 has been amended as shown above, and claim 4 was cancelled above. The claims were amended to more fully clarify the invention. No new matter has been added by the amendments above. Favorable reconsideration is respectfully requested in light of the above amendments and the following comments.

The Examiner objected to claims 2 and 4. Claim 2 included a typographical error which was corrected. Claim 4 has been cancelled herein.

The Examiner rejected claims 2-6 under 35 U.S.C. § 103(a) as being unpatentable over Boyer et al. (U), Boyer et al (V) in view of Horikoshi et al., Colaruotolo et al. (B), Boyer et al. (IDS), Takowa et al. (IDS), and Jones (IDS).

The Examiner asserts that based on the teachings of the above references, one of skill in the art would have been motivated to further select and acclimate the alkaliphilic bacteria isolated for sewage to be best suited for alkaline wastewater treatment, specifically, be suitable at high pH and be able to produce acids because it is known in the art that bacteria may be selected and acclimated to be used in environments for a specific purpose, such as in alkaline condition, and for producing acid to neutralize the alkaline condition.

Applicants respectfully disagree with the Examiner and assert that the Examiner has failed to make out a *prima facie* case of obviousness. In order to establish *prima facie* obviousness, three basic criteria must be met, namely: (1) there must be some suggestion or motivation to combine the references or modify the reference teaching; (2) there must be a reasonable expectation of success; and (3) the reference or references when combined must teach or suggest each claim limitation. Applicants submit that the Office Action failed to state a *prima facie* case of obviousness, and therefore the burden has not properly shifted to Applicants to present evidence of nonobviousness.

Applicants assert that the Examiner has failed to make out a *prima facie* case of obviousness because the combination of the references do not teach or suggest each claim

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limitation. Step (j) recites "mixing said selected bacterial strains from step (i) to obtain mixed bacterial suspension". Applicants respectfully assert that nothing in the references teaches or suggests the combination of bacterial strains for wastewater treatment, and that therefore, the Examiner has not made out a *prima facie* case of obviousness.

Furthermore, even if the Examiner had made out a *prima facie* case of obviousness, Applicants respectfully assert that the invention is non-obvious because the results obtained by Applicants are unexpected. When the combination of the bacterial strains were utilized to reduce the pH of alkaline wastewater, the pH drop that was observed was higher than expected by the two strains alone. The step of combining the two strains created an unexpected result, and therefore, even if the Examiner had made out a *prima facie* case of obviousness, the results of that added, and non-obvious step were unexpected.


Based on the above comments, Applicants respectfully request that the rejection under 35 U.S.C. § 103(a) be withdrawn.

Conclusion

In view of the amendments and comments presented herein, favorable reconsideration in the form of a Notice of Allowance is respectfully requested.

Respectfully submitted,
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Marked up version of Claims**In the Claims**

Please cancel claim 4 without prejudice or disclaimer of the subject matter contained therein. Please amend claim 2 as follows:

2.) (Amended) A process for the preparation of microbial composition useful for the neutralization of alkaline waste-waters[, which comprises] comprising:

(a) isolating the bacterial strains *Bacillus alkalophilus* and *Biacillus* sp. from sewage by standard method;

(b) inoculating [the] said individual bacterial strains in an Alkaline Bacillus broth medium containing $MgSO_4$;

(c) growing [the] said individual bacterial strains for 16-24 hrs;

(d) inoculating [the] said individual bacterial strains from step (c) in a modified Tryptone Soya Broth medium, having original pH value about 7.0, [the] said pH value of the medium being adjusted to different pH values ranging between 8.0-11.0, using Tris-HCl buffer and NaOH- Na_2CO_3 buffer or $NaHCO_3$ - Na_2CO_3 buffer under sterile conditions[:];

(e) growing [the] said individual bacterial strains obtained from step (d) in modified Tryptone Soya Broth medium for 16-24 hrs. at different pH values ranging from 8.0-11.0 to select[ed] the[4] bacterial strains growing at pH 11.0 and the remaining bacterial strains unable to grow at pH 11.0 being acclimatized at pH 11.0[:];

(f) inoculating [the] said selected acclimatized individual bacterial strains from step (e) in a modified Nutrient Broth medium, having pH values about pH 7.0, [the] said pH being adjusted to different pH values ranging from 9.0-11.0 using NaOH- Na_2CO_3 buffer or $NaHCO_3$ - Na_2CO_3 buffer under sterile conditions;

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(g) adding a dye, phenol red indicator and optionally 1% carbohydrate to [the] said inoculated medium of individual bacterial strains obtained from step (f) to observe the change in colour for acid production and to identify the acid producing strains at pH9.0-11.0;

(h) growing [the] said inoculated bacterial strains obtained from step (g) for a period of at least 2 days and thereafter observing acid production by the change in colour of phenol red in [the] said medium from red to orange, orange to yellow and by measuring the decrease in pH of [the] said medium;

(i) selecting the acid producing bacterial strains at pH 11.0;

(j) mixing [the] said selected bacterial strains from step (i) to obtain mixed bacterial suspension;

(k) centrifuging the mixed suspension of bacterial strains obtained from step (j) at 8,000-12,000 rpm to obtain pellet;

(l) washing the obtained pellet from step (k) by suspending the pellet in triple distilled water and re-centrifuging at 8,000-12,000 rpm; and

(m) collecting the pellet from step (l) and lyophilizing the obtained pellet to store at 1 to 4° C for longer use[;].

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